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Conventional risk factors and DNA Repair Efficiency in Coronary Artery Disease

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ABSTRACT
Coronary artery disease (CAD) is a multifactorial disease caused by the interplay of environmental risk factors with multiple predisposing genes. Many studies were conducted to evaluate the role of conventional risk factors in CAD patients of Kerala. No systematic studies were conducted to assess the alteration in DNA repair efficiency in patients with conventional risk factors associated with CAD. The present study was undertaken to assess and to correlate the role of DNA repair efficiency with the conventional risk factors of CAD. One hundred and twelve clinically proved patients with fifty age and sex matched control subjects were included in this study. Detailed clinical characteristics were recorded using proforma. Peripheral blood lymphocyte micro culture was performed as described by Moorhead (1960) for determining the in vitro mutagen sensitivity. The mean number of bleomycin-induced breaks per cell was calculated WHICH is an indicator of mutagen sensitivity and DNA repair efficiency. 't' test, chi-Square test and logistic regression analysis were performed for data analysis. The result was presented as odds ratio (OR) with 95% confidence intervals. Smoking, alcoholism, diabetes, hypertension and family history of CAD were found to be significantly associated with CAD. Various risk factors like smoking, diabetes mellitus, dyslipidemia and hypertension was found to be significantly associated with increase in mean b/c value. This study clearly indicates that the mean b/c value was higher in subjects with abnormal risk factors like smoking, alcoholism, diabetes and hypertension.

Keywords: Coronary artery disease; DNA damage; DNA repair efficiency; in vitro mutagen sensitivity analysis; mean break per cell (b/c) values

INTRODUCTION

Coronary artery disease (CAD) is the pivotal disease entity in terms of both morbidity and mortality in the entire world population. CAD is epidemic in India and it is one of the major causes of disease-burden and deaths. Mortality data from the Registrar General of India shows that cardiovascular diseases are the major cause of death in India [1]. The huge burden of CAD in the Indian subcontinent is the consequence of the large population and the high prevalence of CAD risk factors [2]. Conventional risk factors like hypertension, hyperlipidemia, diabetes mellitus, family history, smoking etc contribute only 50% of the total risk of CAD [3]. This suggests that the major CAD risk factors still to be identified. The study of these risk factors is important since the ability to accurately predict the CAD risk of a specific individual based on his or her conventional risk factor profile is limited [4].
DNA damage has been found as an emerging risk factor to play an important role in atherosclerosis and coronary artery disease [5]. DNA damage is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus [6]. Usually the cells have repair mechanisms that identifies and correct such defects. Therefore DNA repair is essential to an individual's ability to respond to the damage caused by environmental mutagens and reactive cellular metabolites [7]. Inter individual variability in DNA repair capability is an important factor influencing the risk of CAD. Hsu et al [8] developed an assay in which the frequency of chromatid breaks induced by bleomycin in cultured lymphocytes in vitro was quantified as a combined measure of mutagen sensitivity and DNA repair capacity; the number of bleomycin-induced breaks per cell was used to identify sensitive subjects. In an earlier study by Simon et al [9] the mean break per cell (b/c) values which is an indicator of decreased DNA repair efficiency was found to be significantly increased in CAD patients ($P < 0.05$). A systematic approach to evaluate the impact of various lifestyle risk factors on the molecular mechanisms in CAD is not fully understood.
No serious attempts were made earlier to correlate DNA repair efficiency with conventional risk factors of CAD. Hence present study was undertaken to assess alterations in DNA repair efficiency in patients with conventional risk factors associated with CAD. An attempt was also made to correlate the DNA repair efficiency with risk factors such as obesity, diabetes, hypertension and also the habit of smoking and alcoholism.

MATERIALS AND METHODS

One hundred and twelve clinically proved CAD patients from Pushpagiri Heart Institute and fifty healthy age and sex matched controls were included in this study. Detailed clinical characteristics were recorded using proforma. Ethical approval from the Institutional ethics committee, Pushpagiri Institute of Medical Sciences and Research Centre and informed consent were obtained. Three ml of venous blood was collected and used for peripheral lymphocyte culture and in vitro mutagen sensitivity assay for determining DNA repair efficiency. Peripheral blood lymphocyte micro culture was performed as described by Moorhead [10] for determining the in vitro mutagen sensitivity. Bleomycin (mutagen) was added to induce

chromosomal breakage, chromatid breaks were scored and the mean number of breaks/cell (b/c) was calculated according to the method of Hsu et al [8]. 'T' Test, Chi-square test and logistic regression analysis were performed for data analysis. The result was presented as odds ratio (OR) with 95% confidence intervals.

RESULT

Association between risk factors among the test and control with coronary artery diseases is done by Chi square test. Smoking, alcoholism, diabetes, hypertension and family history of CAD were found to be significantly associated with CAD (table 1).

Subjects having the habit of smoking have an increased chance of developing CAD than subjects without smoking (Odd's Ratio = 11.918; Confidence interval = 4.020 - 35.334). Alcoholism is another risk factor for developing CAD (Odd's Ratio = 5.609; Confidence interval = 2.065 - 15.236). Subjects with hypertension have 13.51 times more risk for developing CAD than those without hypertension. Subjects with diabetes have 7 times higher risk than subjects without diabetes for developing CAD. Family history of CAD is a contributing factor for CAD (up to 3.526 times).

Logistic regression analysis for coronary artery disease was performed for the following lifestyle / risk factors viz smoking, alcoholism, diabetes, hypertension, dyslipidemia, family history of CAD and the results were given in the table 2. The variable which is found to be highly significant in the logistic regression analysis was hypertension.

Distribution of mean b/c value with risk factors like smoking, alcoholism, diabetes, hypertension, dyslipidemia, and family history of CAD is given in table 3. The mean b/c value was higher in subjects with abnormal risk factors.

Association between risk factors among the test and control with mean b/c value is done by Chi square test is given in table 4. Smoking, diabetes, hypertension and dyslipidemia were found to be significantly associated with high b/c value (≥ 0.8000) (table 3).

Logistic regression analysis for high (sensitive) mean b/c value (>0.8000) was performed for smoking, diabetes, hypertension and dyslipidemia and the results were given in table 5. Mean b/c value > 0.8 (sensitive) is significant for the variables in the logistic regression analysis were smoking (5.465 times), diabetes (4.220 times) and hypertension (4.679 times).

DISCUSSION

Coronary artery disease is a multifactorial disease which is caused by both genetic and environmental factors. Many studies were conducted to evaluate the role of conventional risk factors like hypertension, hyperlipidemia, diabetes mellitus, family history, smoking etc in CAD patients of Kerala. But these factors contribute only 50% of the total risk of CAD (Muhlestein, 2002) [11].

Cigarette smoking constitutes the single most important, independent and effective risk factor of atherosclerosis as per many previous studies. It is well known that the risk of smokers developing coronary heart disease is at least 2–4 times of that seen in nonsmokers [12]. Many prospective studies show an inverse association between moderate drinking and risk of heart attack, ischemic (clot-caused) stroke, peripheral vascular disease, sudden cardiac death, and death from all cardiovascular causes[13]. The present study is in well agreement with the previous reports, as we also found a strong correlation between CAD and various risk factors like smoking and alcoholism.

A large proportion of premature CAD cases do show evidence for a positive family history of CAD [14], [15] which is in agreement with the present study. In the present study high prevalence of positive family history of CAD in the test subjects clearly indicates that family history of CAD is a high risk for developing CAD in the family members (Odd's Ratio = 3.526; Confidence interval = 1.514 - 8.210).

The risk for CAD is greater among diabetic subjects than among no diabetic subjects [16],[17], [18]. According to Baguet et al [19] among the numerous risk factors associated with coronary artery disease hypertension plays a major role due to its high frequency and physiopathogenesis. The study by Sawant et al [20] revealed the prevalence of hypercholesterolemia, hypertriglyceridemia and abnormally high LDL cholesterol and low HDL cholesterol levels which are well-known risk factors for cardiovascular diseases in all age groups. The higher the number of risk factors, higher will be the chance of developing CAD. In the present study smoking, alcoholism, diabetes, hypertension and family history of CAD were found to be significantly associated with CAD which is in agreement with the above studies.

DNA damage has been found as an emerging risk factor to play an important role in coronary artery disease and when the DNA repair mechanisms break down or overwhelmed, a person may develop heart disease. Therefore DNA repair is essential to an individual's ability to respond to damage caused by environmental mutagens and reactive cellular metabolites [7].This study clearly indicates that the mean b/c value was higher in subjects with abnormal risk factors which are in agreement with the previous studies by Andreassi [6]. Smoking can cause oxidative DNA damage, inhibit DNA repair, and induce the production of advanced glycation end products, which themselves cause DNA mutation [21]. In an earlier study by Simon et al [22] DNA damage is increased with risk factors, mainly smoking. However, in the

current study, majority of male subjects in the test group were smokers, and found a statistical correlations between smoking and chromatid breaks per cell. The mean b/c value of the CAD subjects with smoking were significantly higher than without smoking ($p<0.05$). This is well in agreement with all the earlier studies.

Topinka et al [23] observed a connection of DNA damage and repair and the possible role of active oxygen species in cell damage caused by ethanol. This present study could not observe a significant difference in mean b/c value in subjects with and without alcoholism.

DNA damage is the initiation step of diseases of genetic origin. DNA damage is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus [6] Nishtha et al [24] observed that extent of DNA damage is more in diabetic rabbits as compared to the non-diabetic or antioxidant supplemented group. In this study the mean b/c value was higher in subjects with diabetes and the difference was significant. Diabetes was found to be significantly associated with increase in mean b/c value (P= .000). These findings are well in agreement with all the earlier studies.

Subash et al [25] observed that DNA damage caused by ROS occurs more in newly diagnosed essential hypertensive patients than in persons with normal blood pressure and that antihypertensive drug significantly reduces DNA damage in essential hypertension in vivo. This present study observed a significant increase in the mean b/c value among the test subjects with hypertension than those without hypertension. Hypertension was found to be significantly correlated with increase in mean b/c value (P= .000).

Martinet et al [26] observed that in cholesterol-fed rabbit's plaques also manifest DNA damage associated with up regulation of DNA repair enzymes. In addition, repair pathways started to decline progressively when cholesterol-fed animals were placed on a normal diet. These studies demonstrate that DNA damage and activation of repair pathways occur in atherosclerosis, and are also reversible, at least in the early stages of atherosclerosis [27].In this study low HDL cholesterol is significantly associated with high mean b/c value which reflects increased DNA damage and deficient DNA repair efficiency.

From these findings it can be concluded that CAD is the result of multifactorial influences, some are modifiable and others are not. The increase in the prevalence, morbidity and mortality can be strongly attributed to the changes in lifestyle like habit of smoking and/ or alcoholism. Diabetes mellitus and hypertension are the major risk factors for CAD. Glycemic control, blood pressure control in diabetes mellitus and hypertension will reduce the risk of CAD. These lifestyle modifications will reduce the risk of CAD by reducing DNA damages and improving DNA repair efficiency.

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Table 1.
Odds's ratio, 95% confidence interval and significance for test and control group according to lifestyle / risk factors for Coronary Artery Diseases.

Conventional Risk Factors	Odds's Ratio	Confidence interval	$\chi 2$	P
Smoking	11.918	4.020-35.334	27.091	0.000
Alcoholism	5.609	2.065-15.236	13.365	0.000
Hypertension	13.514	5.532-33.014	41.505	0.000
Dyslipidemia	6.097	2.929-12.689	25.645	0.000
Diabetes	7.000	2.584-18.962	17.719	0.000
Family h/o CAD	3.526	1.514-8.210	9.18	0.000

Table 2.
Logistic Regression analysis showing correlation between risk factors and Coronary Artery Disease

Conventional Risk Factors	B	S.E.	Wald	df	Sig.	Exp(B)
Smoking	1.337	1.518	.776	1	.378	3.809
Alcoholism	3.180	1.905	2.786	1	.095	24.040
Diabetes	2.876	1.522	3.568	1	.059	17.735
Hypertension	3.077	1.098	7.855	1	.005	21.687
Family history CAD	2.950	1.572	3.524	1	.060	19.112
Dyslipidemia	2.271	1.181	3.694	1	.055	9.685

Variable(s) entered on step 1: Smoking, Alcoholism, Diabetes, Hypertension, Family history of CAD and mean b/c value.

Table 3.
Distribution of Mean b/c value according to risk factors in test and control group

Risk Factors		Mean b/c value	
		Control	Test
Family History of CAD	Yes	0.6693±0.035	0.8046±0.069
	No	0.7057±0.0451	0.7989±0.071
Alcoholism	Yes	0.6981±0.023	0.8030±0.071
	No	0.7007±0.047	0.8012±0.069
Smoking	Yes	0.7055±0.018	0.8221±0.062
	No	0.6993±0.047	0.7818±0.071
Dyslipidemia	Yes	0.7014±0.042	0.8616±0.054
	No	0.6901±0.031	0.7977±0.068
Hypertension	Yes	0.6960±0.0664	0.8119±0.068
	No	0.7005±0.042	0.7812±0.069
Diabetic	Yes	0.7012±0.046	0.8303±0.059
	No	0.6876±0.028	0.7805±0.07

Table 4.
Association between risk factors among the test and control with mean b/c value

Variables	Chi-square	df	p
Smoking	22.371	1	.000
Diabetes	23.431	1	.000
Hypertension	25.153	1	.000
Dyslipidemia	9.885	1	.002
Overall Statistics	66.098	11	.000

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Table 5.
Logistic Regression analysis showing correlation between risk factors and Mean b/c value
Variable(s) entered on step 1: Diabetes, Hypertension, Smoking and Dyslipidemia

	B	S.E.	Wald	df	Sig.	Exp(B)
Smoking	1.698	.537	9.985	1	.002	5.465
Diabetes	1.440	.456	9.963	1	.002	4.220
Hypertension	1.543	.481	10.299	1	.001	4.679
Dyslipidemia	.438	.485	.818	1	.366	1.550